758BRI MEIER

1003539157

Source: https://www.industrydocuments.ucsf.edu/docs/jzyf0000

THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

P = 5

Application For Renewal of Research Grant

(Use extra pages as needed)

First Renewal ☒ Second Renewal ☐ Date: 12 February 1973

1. Principal Investigator (give title and degrees): Hans Meier, D.V.M., Dr. med. vet., M.R.S.H., Senior Staff Scientist, Richard R. Fox, Ph.D., Staff Scientist

2. Institution & address: The Jackson Laboratory, Bar Harbor, Maine 04609

3. Department(s) where research will be done or collaboration provided: Hamilton Laboratory, West and Virus-Leukemia Laboratory

4. Short title of study: Oncogenesis in the rabbit: genetic susceptibility, vertical transmission of virus and environmental influences

5. Proposed renewal date: 1 July 1973

6. How results to date have changed earlier specific research aims: The results obtained to date have not changed our original basic aims. However, several new approaches are proposed.

7. How results to date have changed earlier working hypothesis: Our earlier working hypothesis remains unchanged.

1003539158

Source: https://www.industrydocuments.ucsf.edu/docs/jzyl0000

8. Any additional facilities now required? Describe briefly: New stainless steel cages are required for replacement of old galvanized cages.

9. Any changes in personnel? Append biographical sketches of new key professional personnel: No personnel changes are contemplated.

10. Append outline of experimental protocol for ensuing year. See attached protocol.

11. List publications or papers in press resulting from this or closely related work. (append reprints or manuscripts not previously sent). See attached.

12. Summary progress report (append in standard form as separate document, unless recently submitted). Included in objectives or specific aims.

10035391S9

Source: https://www.industrydocuments.ucsf.edu/docs/zyld0000

13. Budget for the coming year:

A. Salaries (give names or state "to be recruited")	% time	Amount
Professional (give % time of investigator(s) even if no salary requested)		
Hans Meier, Project Director	5	-
R.R. Fox, Project Co-Director	20	$ 3,945
Technical		
D.D. Crary, Professional Assistant	10	1,065
R.F. Norberg, Professional Assistant	15	1,250
Eugene Farrin, Research Assistant	20	1,500
Animal Caretaking		4,000
Secretarial Assistance		500
	Sub-Total for A	$12,260
Employee benefits: 17.5%		2,145
B. Consumable supplies (by major categories)		
Chemicals, glassware, instruments		1,600
Food and bedding		1,700
	Sub-Total for B	$ 3,300
C. Other expenses (itemize)		
Domestic travel to scientific meetings (2 investigators)		500
Publication costs		300
Art, Photo, and Replicating Service		200
	Sub-Total for C	$ 1,000
	Running Total of A + B + C	$16,560
D. Permanent equipment (itemize)		
Thirty (30) Bussey Model 1491S cages		1,800
	Sub-Total for D	$ 1,800
E. Indirect costs (15% of A+B+C)	E	$ 2,805
	Total request	$23,310

10035391160

Source: https://www.industrydocuments.ucsf.edu/docs/jzfd0000

14. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
ncogenesis in the rabbit: genetic usceptibility, vertical trans-ission of virus, and environ-ental influences ee attached.	The Council for Tobacco Research, U.S.A.	$21,330*	6/1/72-5/31/73

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Natural occurrence of RNA tumor viru (genomes) and host-gene con l of their expressions See attached.	National Cancer Institute	$412,529	5/1/73-4/30/74

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Hans Meier

Signature Hans Meier Date 5/12/73

Telephone 207-288-3373 273
Area Code Number Extension

Checks payable to The Jackson Laboratory
tin C. Carter, Financial Manager

Mailing address for check:
Bar Harbor, Maine 04609

Responsible officer of institution

Typed Name Austin C. Carter

Title Financial Manager

Signature [signature] Date 13 May 73

Telephone 207-288-3373 227
Area Code Number Extension

10035391

Source: https://www.industrydocuments.ucsf.edu/docs/jzyl0000

14. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
tural occurrence of RNA tumor vir- (genomes) and host-gene control their expressions	National Cancer Institute	$395,064	5/1/72-4/30/73
reditary diseases and variations the rabbit	NIH General Research Support grant to the Jackson Laboratory	63,836*	7/1/72-6/30/73
bbit inbred and mutant stocks source	NIH Division of Research Resources	25,000*	1/1/73-12/31/73

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
reditary diseases	National Institute of General Medical Sciences	$ 50,487*	4/1/73-3/31/74
bbit inbred and mutant stocks source	NIH Division of Research Resources	90,015*	1/1/74-12/31/74
nes, viruses, and cancer	National Cancer Institute	69,327*	4/1/73-3/31/74

*Direct costs

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Checks payable to

Mailing address for checks

Principal investigator

Typed Name

Signature Date

Telephone
Area Code Number Extension

Responsible officer of institution

Typed Name

Title

Signature Date

Telephone
Area Code Number Extension

10035391/2

Source: https://www.industrydocuments.ucsf.edu/docs/jzyl0000

Details of Experimental Design and Procedures (from page 2):

Introduction and background. In our applications (1970-72) for CTR Research Grant 758, we stated the aims of our studies in detail. Clearly, the work that we proposed in our 1972 application could not be completed within the period of the 1 year for which we received funding; at least 2 additional years of work and support are required. Thus, this application is for continuance of studies currently underway.

The overall goals of our studies remain basically as proposed previously:

(1) We have observed 75 cases of lymphosarcoma in a small breeding colony of strain WH rabbits within a few years, and affected animals of both sexes were found in each of several generations. Because of the unusual aggregation of cases of lymphosarcoma among WH rabbits, we wish to investigate the host genetic factors conferring susceptibility to lymphosarcoma, the mode of inheritance or transmission, the possibility of a vertically transmitted virus, and the environmental influence that may modify incidence and pathogenesis of lymphosarcoma.

(2) Another strain of rabbits, strain X, which is genetically related to the WH strain, is characterized by a high incidence of immune hemolytic anemia (60 cases); thymoma occurs as well. We wish to determine the pathogenesis of both immune hemolytic anemia and thymoma, and investigate their possible etiological relationship.

We want to find out the mode of inheritance or transmission of immune hemolytic anemia and thymoma in strain X rabbits so that we may evaluate the possibility of a common hereditary basis for all conditions in both strain X and WH, since the two strains are genetically related. The various clinical or phenotypic expressions probably derive from differences in the genetic background of the two strains.

(3) We believe that all three conditions are caused by a vertically transmitted virus analogous to the C-type RNA viruses occurring in a number of vertebrates, including man. The outcome of viral-host interaction depends to major degrees on host genetic factors, but it may be modified by environmental influences.

Scientific progress during tenure of current grant. A brief background and resume of work accomplished this far is pertinent to the studies proposed in this application. Detailed reports of our accomplishments have been submitted (Progress Reports 1, 2, and 3).

(1) Hereditary lymphosarcoma in strain WH rabbits.

(a) Genetic and pathologic studies. In 1962, we first observed a lymphosarcoma in the WH strain of rabbits at the Jackson Laboratory. Since 1965, we have observed 74 more lymphosarcomas in this strain. Because of this unusual aggregation of lymphosarcomas, we have analyzed the pedigrees of the affected animals. Lymphosarcoma in the WH strain of rabbits was found to have a hereditary basis. The inheritance indicated an autosomal recessive gene conferring susceptibility to lymphosarcoma. We designated this gene ls. Our data

10035391163

Source: https://www.industrydocuments.ucsf.edu/docs/jqjl0000

are compatible with the concepts of genetic susceptibility to lymphosarcoma and of vertical transmission of a virus. Affected rabbits usually died between the age of 5 and 13 months. The neoplastic involvement of lymphoreticular and other organs, especially kidneys, corresponded to a pattern observed in lymphosarcoma of other domestic animals. Specifically, it resembled in many ways visceral lymphosarcomatosis of cats that has been proved unequivocally to be caused by feline leukemia virus. Similarities between rabbit and cat lymphosarcomas include the sites of onset, distribution of the neoplastic lesions, and the finding of a predominantly aleukemic hemogram. However, in rabbit lymphosarcomas, we often found a relative increase in lymphoid cells including both immature and atypical forms (Fox et al., 1970).

If a C-type RNA virus is demonstrated, the ls gene may confer susceptibility to malignant transformation of lymphoreticular tissues. We have found a number of genes in mice that enhance oncogenesis, especially leukemogenesis, but that do not influence the presence or absence of either murine leukemia virus (MuLV) or MuLV antigens. We are now studying the possible viral etiology of rabbit lymphosarcoma. Further, we are investigating possible linkage relationships between ls and other genes in strain WH and related rabbits.

Rabbits are of considerable value in biomedical research because of the vast amount of morphologic, physiologic, and biochemical data available on rabbits, the simplicity of rabbit care and breeding, and their large size. The finding of lymphosarcoma and its hereditary basis provides a new and important model for studies of the pathogenesis of neoplasia. The rabbit colonies at the Jackson Laboratory are free of Shope papilloma and fibroma- and myxomatosis viruses. Except for a small number of epithelial tumors, which we described (Meier et al., 1970, Fox et al., 1971), most tumors in our rabbits have been of lymphoid tissue origin, i.e., lymphosarcoma and thymoma.

(b) Experimental transmission of lymphosarcoma. Cell-free extracts including Moloney concentrates from lymphosarcoma tissue were inoculated by various routes into young adult, newborn, and fetal WH rabbits. No successful transmission has as yet occurred. However, we have successfully transmitted lymphosarcoma with cell suspensions. Whereas young adult WH rabbits are resistant, fetal animals are sensitive to growth of tumor-cell inocula. Newborn rabbits are partially resistant; a single animal developed a tumor at the site of inoculation. In contrast, all four fetuses inoculated with cells at 25 days of pregnancy developed generalized lymphosarcoma. One animal died 4 weeks after birth; the remaining three were killed at 4 weeks of age and revealed typical lymphosarcomas.

(c) Electron microscopic search for complete type C RNA tumor virus(es). Extensive electron microscopic studies of fresh and tissue culture materials from normal and lymphosarcoma-afflicted rabbits of strain WH included the following: thymuses, popliteal and mesenteric lymph nodes, spleens (6 specimens of each), kidneys (5), livers (4), adrenals (2), ovary, testis, and bone marrow (1 each); in addition, we studied fresh liver tissue from seven embryos of 17, 18, and 24 days of fetal age. Tissue cultures consisted of primary, secondary, and established lines (P_3 - P_{20}) from 17- to 24-day whole embryos and lymphosarcoma-affected organs (thymus, spleen, kidney) from adult rabbits. No type C particles were observed, indicating that the viral genome is present in an incomplete or covert form. A single liver sample from a

1003539164

Source: https://www.industrydocuments.ucsf.edu/docs/zyl0000

lymphosarcomatous rabbit yielded intracisternal (immature type C or A particles?) particles that we do not consider characteristic; no membrane-budding particles could be detected.

The addition to both embryo- and lymphosarcoma-tissue cultures of carcinogenic chemicals has not yet yielded transformed cells; neither has radiation nor iododeoxyuridine (IUdR) and bromodeoxyuridine (BUdR) induced complete infectious type C virus.

(d) Attempts at induction of tumors and virus rescue. In an attempt to induce complete virus formation, we have tried to induce tumors by various means including body irradiation, carcinogens (DMBA, MCA), injections of partially transformed cells, and inoculation of murine type C pseudotype viruses (MSV, RLV, and KiMSV).

The rabbit appears to be extremely resistant to tumor induction by such exogenous means; hence, we are now studying the inducibility, or lack of it, of arylhydrocarbon hydroxylase, an enzyme which converts hydrocarbon compounds to the proximate carcinogen (hydroxylated compounds, epoxides).

Complete infectious virus is not a prerequisite to tumorigenesis; however, isolation of a virus is necessary for the preparation of antisera specific for the rabbit species-specific group-specific antigen (gs-1). In view of our failure so far to induce infectious virus, we have concentrated our efforts on the demonstration of the interspecies-specific group-specific antigen (gs-3) which cross-reacts with specific antisera made to other mammalian type C RNA tumor viruses, e.g., murine and feline viruses.

(e) Search for the interspecies antigen (gs-3) of type C RNA tumor virus. Complement-fixation (CF) tests for the interspecies group-specific antigen (gs-3) of type C RNA tumor virus in strain WH yielded positive results with antiserum MSV I-13 and anticomplementary reactions with goat anti-feline leukemia virus antiserum, IS-8. The two antisera give reactions to the respective viral gs-1 and gs-3 antigens by immunodiffusion; in addition, each antiserum reveals an unidentified line probably due to a viral coat- or tissue antigen. Since rabbit tissues cannot react with either this antigen or the species-specific gs-1, the CF reaction obtained with MSV I-13 must be specific for gs-3.

Similarly, we found, by indirect peroxidase-labeling, specific gs-3 reactivity of several tissue culture rabbit tumor lines. Gs-3 reactions were also obtained upon immunodiffusion from both normal and tumorous tissues including the thymus, lymph nodes, gastrointestinal tract, and gonads. The immunodiffusion studies were done in collaboration with Drs. Stephen Oroszlan and Raymond V. Gilden, Flow Laboratories, Inc. Studies are now in progress utilizing the radioimmune (precipitation) assay (RIPA or RIA) developed by Dr. Wade Parks (Viral Carcinogenesis Branch, National Cancer Institute) and Dr. Gilden, respectively, and designed to specifically reveal gs-3; this test is the most refined and is several fold more sensitive than both the direct CF- and immunodiffusion tests. Generally, precipitation of less than 90% in the RIPA is considered positive. Thus, unless an anticomplementary reaction is occurring, several of the samples tested are reproducibly positive including skeletal muscle. In order to definitively rule out a nonspecific effect, assays of

1003539195

Source: https://www.industrydocuments.ucsf.edu/docs/yldf0000

additional samples, including normal rabbit tissues are anticipated.

(f) Assays and distribution of DNA-polymerases in rabbit tissues. RNA tumor virus(es) contain an enzyme, RNA-dependent DNA polymerase, that transcribes DNA from an RNA template. The role of this enzyme has not as yet been established, i.e., whether or not it plays a part in transformation or may be essential for a transformed state.

In collaboration with Drs. Masa Hatanaka and R. V. Gilden, Flow Laboratories, we assayed organs of normal, azathioprine (immuran)-treated and lymphosarcoma-afflicted rabbits. Most organs, both normal and malignant, revealed enzyme activity. Thymuses, major mesenteric and popliteal lymph nodes, and gastrointestinal tract have the highest specific activity of RNA-dependent DNA polymerase suggesting the common nature or origin of the lymphopoietic systems. Also, the data taken together with our serological findings verify the presence of an RNA viral genome in WH rabbits. It is also present in other incipient inbred strains of rabbits, i.e., IIIDw and a hybrid between two strains (III_{mo}/III_c).

(g) Pedigree analyses for lymphosarcoma- and immune hemolytic anemia susceptibilities. We have now observed 75 cases of lymphosarcoma in strain WH and seven cases in genetically related rabbits of strain AX. Autoimmune hemolytic anemia occurred in 60 rabbits of strain X; in addition, four cases were found in strain AC which is in part derived from strain X. In fact, all affected individuals in the four strains are genetically related and trace back to a common ancestor, X974. Thus, we suspect that the two different syndromes, each caused by an autosomal recessive gene, ls and ha, respectively, may indeed be manifestations of the same gene with the phenotype dependent upon the remainder of the host genotype. To either confirm or rule out this possibility, we are now awaiting results of sufficient matings between ls/+ and ha/+ heterozygotes.

Certain of these aspects including our pathological and hematological findings in strain WH and X afflicted with lymphosarcoma and immune hemolytic anemia have been presented at the First Annual Symposium of Hycel, Inc. (Fox, 1972) and in two book chapters (Fox, 1973; Lindsey and Fox, 1973).

(h) Myeloid leukemia in strain III. We have now found the first case of myeloid leukemia in the rabbit. It occurred in a 13-1/2 month old male of subline III_{ep}. Its features are distinct from hereditary lymphosarcoma by cell type, organ involvement, and distribution of tumors. Studies are in progress to determine whether this case of myeloid leukemia is of hereditary origin. Also, we plan to determine whether an oncogenic type C RNA viral genome is involved in this, as well as in lymphosarcoma of WH rabbits (Meier et al., 1972).

(i) Genetic predisposition to tumors in the rabbit. Our analysis of genetic factors has been presented in Progress Report 2, and a paper describing our findings has been published (Fox et al., 1971).

(2) Hereditary hemolytic anemia associated with thymoma in strain X rabbits. Autoimmune (Coombs-positive) hemolytic anemia occurs with high frequency in rabbits of strain X. This condition is rapidly fatal with a mean sur-

100539166

Source: https://www.industrydocuments.ucsf.edu/docs/jzyf0090

vival time of about 5 months. Sometimes it is associated with thymic hyperplasia and thymoma. The gene conferring susceptibility, designated ha, may be identical with that causing lymphosarcoma susceptibility and assigned the gene symbol, ls, in WH rabbits. Strain X and WH are closely related genetically, and a common gene responsible for all conditions may have phenotypic expressions that are dependent upon the remainder of the genotype. We are also considering the possibility that a vertically transmitted virus similar or analogous to the C-type RNA virus of mice is of etiological importance in addition to gene(s) conferring susceptibility (Fox et al., 1971).

Methods and procedures of proposed studies. In our studies of the pathogenesis of lymphosarcoma in WH rabbits and of hemolytic anemia associated with thymoma in strain X rabbits, we wish to achieve an understanding of the underlying mechanisms leading to each disorder in the two strains. No doubt the interactions of host genotype, "environment," and C-type RNA virus, if present, are complex. In the light of our findings to date we are proposing the following studies:

(1) Studies in strain WH rabbits. Apparently complete infectious C-type RNA tumor is absent or occurs only rarely. Screening of tissues from lymphosarcoma-affected, normal, and embryonic rabbits by electron microscopy has not as yet yielded C-type particles. Thus, we are no longer attempting cell-free transmission of lymphosarcoma.

(a) If complete virus is indeed absent, we may (1) induce complete viral synthesis by carcinogenic chemicals, immunosuppressive agents, and various nucleosides, and (2) continue to screen rabbit tissues for the cross-reacting (gs-3) viral group-specific antigen using at least two antisera that can detect the interspecies determinant by gel diffusion.

(b) If complete virus is inducible in any strain of rabbits, its isolation and purification is essential for the production of specific antisera.

Approaches a2 and b have been described in previous applications. In the past year, several new ways and means of tumor-induction in rabbits have evolved that may bring us closer to the successful activation of the putative endogenous C-type viral genome. These are generally relevant to cocarcinogenesis and relate to the following observations and procedures:

(a) Inducibility of aryl hydrocarbon hydroxylase in rabbits. Aryl hydrocarbon hydroxylase (AHH) is representative of a class of oxidative microsomal enzyme systems that hydroxylates a variety of compounds including carcinogenic polycyclic hydrocarbons. A number of pertinent observations now support the view that the metabolism of such carcinogens is related to both their induced carcinogenicity and cytotoxicity. The inducibility of AHH is strain-dependent in mice and hamsters; in fact, it depends on a single autosomal gene. Strains that develop high basal AHH activity in response to treatment with polycyclic hydrocarbons would be expected to produce high levels of reactive intermediates with enhanced carcinogenicity. Although we do not have as yet rigorous data on the inheritance of AHH inducibility in rabbits, various strains differ greatly in their inducibility. Strain III is regularly inducible and thus is an excellent strain for chemical carcinogenesis studies.

10035391167

Source: https://www.industrydocuments.ucsf.edu/docs/gyyk0000

(b) Transplacental carcinogenesis by 1-ethyl-1-nitrosourea. We have found in mice that susceptibility or resistance to 1-ethyl-1-nitrosourea (ENU) is clearly hereditary; these findings are of considerable importance regarding the proposed "direct action" of ENU and, possibly, other nitrosocompounds. It now appears that the outcome of ENU-induced effects most likely depends upon genetically determined differences in the cellular repair mechanism rather than in its metabolism or transplacental uptake, although we found a certain association with the inheritance of AHH inducibility.

Developing embryos are particularly susceptible to ENU, and an inverse relationship exists between its teratogenicity and carcinogenicity. Strain III_{VO} fetuses are highly susceptible to ENU-induced renal tumors when pregnant mothers are treated on day 18 of pregnancy; multiple tubular adenomas and adenocarcinomas develop as early as 40 days after birth. These tumors occur reproducibly and within a limited time, and provide us with an opportunity to search for endogenous rabbit C-type tumor virus(es).

(c) Intratracheal application of 3-methylcholanthrene. We are injecting 3-methylcholanthrene (MCA) intratracheally into both AHH-inducible and non-inducible strains of rabbits. MCA is suspended in a sterile medium of physiological saline and 0.2% gelatin (w/v) to give a concentration of 50 mg MCA/0.5 ml medium per injection. Several injection schedules are used. If presently available animal models are to be effectively applied to studies on the etiology and pathogenesis of human lung cancer, detailed information on the histogenesis of experimentally induced respiratory tract tumors is essential. The tumors that may develop will again be screened for C-type viruses.

(d) Potentiation of methylcholanthrene-carcinogenicity by tobacco condensate. Aqueous extracts of cigarette-smoke condensates have been found to enhance benzpyrene-induced liver AHH and menadione reductase activities in rats. Without benzpyrene, AHH was moderately increased by tobacco condensate, whereas menadione reductase was unaffected. Since we found most strains of rabbits extremely resistant to MCA carcinogenesis, we are administering to various strains of rabbits aqueous extracts of cigarette-smoke condensates in sugared drinking water. After 1 month they are then injected with MCA in trioctanoin. Tobacco condensates are kindly provided by Dr. A. R. Patel of Meloy Laboratories, Springfield, Va., and through the courtesy of Dr. John Kreisher, The Council for Tobacco Research, USA--Inc.

Thus, we aim by various means to induce tumors of both epithelial (pulmonary, renal) and mesenchymal (sarcomatous) types in efforts to simultaneously activate endogenous C-type virus. Radiation-leukemia virus (RadLV) of C57BL/6J mice exemplifies the induction of complete virus in a strain of mouse that ordinarily lacks infectious virus expression. Another example is the induction of the group-specific antigen in negative strains, e.g., C57L/J mice by carcinogenic chemicals; clearly no genetic information for antigen-synthesis is conferred by the chemical.

(2) Studies in strain X rabbits. Information is needed to decide whether (1) the same gene that is responsible for susceptibility to hereditary immune hemolytic anemia also predisposes to thymoma, (2) the gene giving rise to hemolytic anemia or thymoma, or both, in strain X is the same gene that is responsible for lymphosarcoma-susceptibility in WH rabbits, and (3) both hemo-

100539168

Source: https://www.industrydocuments.ucsf.edu/docs/zyb0000

lytic anemia and thymoma are due to an interaction with a vertically transmitted C-type RNA viral genome.

The following procedures should provide the answers sought to the first two questions. Information on the third will result from approaches identical or analogous to those described for WH rabbits.

(a) Is the same gene responsible for both hemolytic anemia and thymomagenesis? We are chronically treating strain X rabbits homozygous for the hemolytic anemia trait (ha/ha) with the immunosuppressive drug, azathioprine (Imuran). Since the hemolytic disease is due to an immune disorder, azathioprine should prevent its occurrence. However, by analogy to NZB mice, it should not interfere with development of thymoma. Thus, in order to establish that the gene is responsible for both conditions, we must be able to selectively induce thymoma upon continued azathioprine therapy.

(b) Are the genes, lymphosarcoma-susceptibility (ls) and hemolytic anemia (ha) identical? In order to answer the question of allelism of ha and ls, F_1-hybrids between respective heterozygotes (ls/+ and ha/+) should yield approximately 25% "abnormal" offspring if the two genes are allelic. We cannot of course decide a priori whether they resemble either of the respective homozygotes (ls/ls and ha/ha) or whether they have clinical features of both.

Genetic studies are time-consuming and require large numbers of animals. Also, we cannot know what the latency period may be for clinical signs to appear in case compound heterozygotes (ls/ha) are obtained.

In past and current breeding experiments we have made the following crosses, ha/ha x ls/+ and ha/+ x ls/+; indeed, several compound heterozygotes have appeared. These findings clearly indicate the identity of ha and ls. In order to obtain a representative spectrum of phenotypic expression of compound heterozygotes, we anticipate a requirement of at least 10 afflicted rabbits.

So far, affected rabbits suffered either from hemolytic anemia or a combination of hemolytic anemia and a lymphoproliferative disorder depending upon the age at which clinical signs appeared.

(3) Both strains of rabbits. Strains WH and X are "partially inbred." The coefficient of inbreeding, which defines that proportion of loci for which the original or base population was heterozygous but which through inbreeding has become homozygous, is approximately 0.7 and 0.8, respectively (Appendix). We estimate, therefore, that each strain may be homozygous for as many as 75% of the initially variable loci and that these loci have been fixed for different alleles in the two strains because of deliberate selection for and maintenance of specific mutant genes.

A population of animals need not be inbred for an analysis of the inheritance of a specific gene or phenotypic trait. The genetic basis of a trait by which two strains differ can be obtained by crosses to obtain F_1-, F_2-, and backcross generations. From populations segregating in Mendelian proportions for recognizable phenotypes, one may estimate the number of gene pairs that

100353169

Source: https://www.industrydocuments.ucsf.edu/docs/xxyl0000

distinguish the two parental strains with respect to the trait in question. Conversely, because there is reduced genetic variability within inbred strains, they are unsuitable for selection experiments unless heterogenous "foundation" populations are synthesized by crossing two or more strains. Since the two rabbit strains are now only "partially inbred," we can select for those genes that produce a particular phenotype of interest, e.g., lymphosarcoma susceptibility and resistance. Thus, in each strain we should be able to produce two or more lines that may vary in tumor development and also a line that is tumor-resistant. These may interact specifically, but variously in studies of chemical or other cocarcinogenesis.

In addition to the 75 cases of lymphosarcoma in strain WH, we have now also found seven cases in genetically related rabbits of strain AX. Also, in addition to the 60 cases of autoimmune hemolytic anemia in strain X, four cases were observed in strain AC, which is in part derived from strain X. In fact, all affected individuals in the four strains are genetically related and trace back to a common ancestor, X974.

We are now looking for circulating antinuclear and anti-DNA antibodies in the various rabbit strains, as well as immune complex disease in biopsies from kidneys. Since lymphoid cell lines maintained in suspension on a gyratory shaker have yielded complete virus from NZB and related mice, we are using analogous conditions for cell lines produced from spleens and lymph nodes.

Significance of this research. The proposed studies relate to an opportunity for the analysis of two major groups of disorders: (1) cancer and (2) autoimmune disease. They do not deal directly with the effects of tobacco, but are clearly relevant to tobacco effects in several indirect ways: (1) neither tobacco or its various chemically-defined components nor all known carcinogenic chemicals provide a host with the genetic information to produce or induce cancer and any other disease, (2) the occurrence of cancer or any non-neoplastic disease is dependent upon the inborn host-genetic regulation of all processes allowing or disallowing it to occur. Thus, an analysis of the hereditary pathways and their acquired modifications through tobacco or other means is fundamental to an understanding of all disease processes. Some of these may be attacked by use and study of the two (ls/ls and ha/ha) mutants of rabbits. Because of their analogies, they may help clarify mechanisms of the respective human disorders and provide basic information about their pathogenesis.

So far, we have made three major findings: (1) the likely identity of the genes conferring susceptibility to both cancer (lymphosarcoma) and immune hemolytic anemia (autoimmune disease), (2) the most probable presence in strains WH, X, and other strains of an endogenous oncogenic C-type RNA tumor viral genome, and (3) strains WH and X have Coombs' autoantibodies. The major significance of our studies lies in their potential relationship:

(1) Hereditary autoimmune disease in strain X. Like human systemic lupus erythematosus (SLE) and the related disease in New Zealand black (NZB) mice and (NZB x NZW) F_1 hybrid mice, hereditary autoimmune disease in strain X is associated with synthesis of various autoantibodies to erythrocytes and various nuclear materials. Some of the possibilities for their occurrence are that: (1) the hosts are unable to properly repair nucleic acids, and altered nucleic acids might be released from cells into the circulation. Such altered nucleic acids might either have an abnormal catabolic fate or antigenicity. This situation would be analogous to the defect in the repair of DNA that occurs in xeroderma pigmentosum. (2) The hosts are deficient in or have abnormal

10035391/70

Source: https://www.industrydocuments.ucsf.edu/docs/jzvl0000

nucleases, and perhaps other catabolic enzymes, that result in the formation of immunogenic breakdown products from spent, autologous cells. (3) The hosts have a defect in some intracellular structure such as the nuclear or plasma membrane resulting in an inability to keep nucleic acids in a proper configuration or an intracellular compartment. And (4), the hosts harbor a virus whose genome is immunogenic because of a different configuration or nucleotide composition than that of the host, thereby terminating tolerance to autologous nucleic acids.

Thus, it seems possible that cells from patients with SLE or diseased NZB mice are variants of one or more of the defects listed. Their detection and evaluation is clearly important in elucidating the pathogenesis of SLE, as well as the catabolism, structure, and repair of nucleic acids in normal cells.

(2) Hereditary lymphosarcoma in strain WH. In addition to autoantibodies to double-helical DNA, DNA-histone complexes, single-stranded DNA, and nucleolar RNA and their complexes, NZB mice also make antibodies to the endogenous C-type RNA tumor virus. The presence of such a genome in both strains WH and X, as well as all other rabbit strains, is most probable. Thus, with genes ls and ha being identical and conferring susceptibility to either lymphosarcoma or autoimmune hemolytic anemia, a common pathogenesis of the two disorders is likely.

We now have evidence for a highly significant association in mice between the endogenous C-type RNA viral genome and tumorigenesis. In fact viral expression in early life is a highly predictable marker for tumorigenesis with advancing age. This expression is host-gene controlled, and relates to tumors of all types, i.e., mesenchymal as well as epithelial tumors, and leukemias as well as solid tumors. Thus, the mechanism for tumorigenesis is hereditary or "built-in," but whether or not tumors will develop depends upon other host-genes as well as environmental factors, be they tobacco or other potential cocarcinogens. The histological type of the target cell, e.g., alveolar, epidermoid, lymphoid, fibroblastic, etc., represents the chief determinant of the pathological kind of tumor that is finally induced. Although this explanation requires ultimate substantiation, it provides the most rational basis for all available tumor data.

10035917

Source: https://www.industrydocuments.ucsf.edu/docs/jzxb0000

Biographical sketches of all principal and professional personnel.

Hans Meier

Born:	21 July 1929, Zurich, Switzerland	
Nationality:	United States citizen	
Education:	University of Zurich (Veterinary Medicine), D.V.M.	1954
	University of Zurich (Pathology) Dr. med. vet.	1957
	Harvard School of Public Health	1957
	Massachusetts Institute of Technology, Alumnus (Biophysical and biochemical cytology, biology)	1959
Honors:	Royal Society of Health (Pharmacology), M.R.S.H.	1963
	Member, Scientific Advisory Board, The Council for Tobacco Research, USA	1971
	Member, Solid Tumor-Virus Working Group, Special Virus-Cancer Program, National Cancer Institute, NIH	1971
Major Interests:	Experimental pathology, cancer	

Positions:

1970-	The Jackson Laboratory, Senior Staff Scientist
1970-	The National Cancer Institute, Consultant
1962-70	The Jackson Laboratory, Staff Scientist
1960-62	The Jackson Laboratory, Associate Staff Scientist
1957-60	Harvard Medical School, Res. Asst. (Pathology)
1958-60	Children's Hospital and Medical Center, Boston, Asst. (Experimental) Pathologist

10035391122

Source: https://www.industrydocuments.ucsf.edu/docs/[illegible]

	1957-60	Children's Cancer Research Foundation Res. Asst., Res. Assoc. (Exp. Pathol.)
	1954-57	Angell Memorial Hospital, Boston, Fellow, Resident, Chief of Pathology
Postgraduate training:	1955-57	Harvard Medical School, Course work in pathology and neuro-pathology
	1957	Harvard University, Summer School of Arts and Sciences, and of Education (Biophysics and radioactive measurements)
	1958	Armed Forces Institute of Pathology (Course in histochemistry)
	1959	Harvard University, Depts. Biology, (Electron microscopy) and Chemistry (Experimental methods in biochem.)

Societies: N. Y. Acad. Sci.; Soc. Exp. Pathol.; Int. Acad. Pathol.; Amer. Soc. Pathol. and Bacteriol.; Cancer Soc.; Histochem. Soc.; Amer. Soc. for Cell Biol.; New England Soc. Pathol.; A.A.A.S.; Conference Res. Workers in Animal Diseases; Basic Research Council, Amer. Heart Ass.; Amer. Ass. Public Health; Royal Society of Health (M.R.S.H.); Soc. Exp. Biol. Med.

100539123

Source: https://www.industrydocuments.ucsf.edu/docs/[illegible]

Richard R. Fox

Born:	12 November 1934, New Haven, Connecticut
Nationality:	United States citizen
Educated:	University of Connecticut (Animal Production) B.S. 1956
	University of Minnesota (Animal Breeding) M.S. 1958
	University of Minnesota (Animal Breeding and Genetics) Ph.D. 1959
	The Jackson Laboratory (Rabbit Genetics), Post-doctoral Fellow 1959-60
Honors:	B.S., with honors and with distinction in Animal Production
	Sigma Xi
	Gamma Sigma Delta
	Alpha Zeta
	Member: Committee on Nomenclature for Inbred Rats, Hamsters, Gerbils, and Rabbits, Nat. Acad. Sci., ILAR
Major interests:	Genetics of the rabbit; quantitative genetics; reproductive physiology; animal husbandry
Positions:	1965- The Jackson Laboratory, Staff Scientist
	1961- University of Maine, Department of Animal and Veterinary Science, Lecturer
	1960-65 The Jackson Laboratory, Assoc. Staff Scientist
Societies:	Amer. Genet. Soc.; Genet. Soc. of Amer.; Amer. Dairy Sci. Ass.; Amer. Ass. Lab. Anim. Sci.; Amer. Soc. Anim. Sci.; Teratology; A.A.A.S.

10035391174

Source: https://www.industrydocuments.ucsf.edu/docs/[illegible]

Relevant publications

MEIER, H., D. D. MYERS, and R. J. HUEBNER. 1969. Genetic control by the hr--locus of susceptibility and resistance to leukemia. Proc. Nat. Acad. Sci., USA 63:759-766.

FOX, R. R., H. MEIER, DORCAS D. CRARY, D. D. MYERS, R. F. NORBERG, and C. W. LAIRD. 1970. Lymphosarcoma in the rabbit: genetics and pathology. J. Nat. Cancer Inst. 45:719-729. (Supported in part by CTR).

HUEBNER, R. J., G. J. TODARO, P. SARMA, J. W. HARTLEY, A. E. FREEMAN, R. L. PETERS, C. E. WHITMIRE, and H. MEIER. 1970. "Switched-off" vertically transmitted C-type RNA tumor viruses as determinants of spontaneous and induced cancer: a new hypothesis of viral carcinogenesis. Proc. 2nd Int. Symp. on Tumor Viruses, Paris, France, No. 183:33-57.

MEIER, H., D. D. MYERS, and R. J. HUEBNER. 1970. Excretion of murine leukemia virus. Nature 226:849-850.

MEIER, H., R. L. HANCOCK, and R. J. HUEBNER. 1970. Effect of poly rI: rC on sRNA methylase in leukemic and normal spleens. Life Sci. 9:641-651.

MEIER, H., D. D. MYERS, R. R. FOX, and C. W. LAIRD. 1970. Occurrence, pathological features, and propagation of gonadal teratomas in inbred mice and the rabbit. Cancer Res. 30:30-34.

MEIER, H., D. D. MYERS, and R. J. HUEBNER. 1970. Differential effect of a synthetic poly-ribonucleotide complex on spontaneous and transplanted leukemia in mice. Life Sci. 9:653-659.

MYERS, D. D., H. MEIER, and R. J. HUEBNER. 1970. Prevalence of C-type RNA virus group-specific antigen in inbred strains of mice. Life Sci. 9: 1071-1080.

FOX, R. R., H. MEIER, DORCAS D. CRARY, R. F. NORBERG, and D. D. MYERS. 1971. Hemolytic anemia associated with thymoma in the rabbit: genetic studies and pathological findings. Oncology 25:372-382. (Supported in part by CTR).

FOX, R. R., H. MEIER, and DORCAS D. CRARY. 1971. Genetic predisposition to tumors in the rabbit. Naturwissenschaften 58:457-458. (Supported in part by CTR).

MEIER, H. and R. J. HUEBNER. 1971. Host-gene control of C-type RNA tumor virus expressions: relevance of studies in inbred mice to cancer in man and other species. Proc. Nat. Acad. Sci., USA 68:2664-2668.

MEIER, H. and R. R. FOX. 1972. Hereditary lymphosarcoma in WH rabbits and hereditary hemolytic anemia associated with thymoma in strain X rabbits. Proc. 5th Int. Symp. Comp. Leukemia Res., Padova, Italy; Bibl. Haemat. (in press). (Supported in part by CTR).

10035391475

Source: https://www.industrydocuments.ucsf.edu/docs/xyvl0000

FOX, R. R. 1972. Rabbit models of human disease and their biochemical characterization. Proc. Hycel Vet. Symp. 1:9-23. (Supported in part by CTR).

MEIER, H., R. R. FOX, and D. D. CRARY. 1972. Myeloid leukemia in the rabbit (Orcytologus cuniculus). Cancer Res. 32:1785-1787. (Supported in part by CTR).

FOX, R. R. 197-. Taxonomy and genetics, Chapter 1. In S. H. Weisbroth, R. E. Flatt and A. L. Kraus (eds.) Biology of the Laboratory Rabbit. Academic Press, New York (in press).

LINDSEY, J. R., and R. R. FOX. 197-. Inherited Diseases and Variations, Chapter 15. In S. H. Weisbroth, R. E. Flatt and A. L. Kraus (eds.) Biology of the Laboratory Rabbit. Academic Press, New York (in press). (Supported in part by CTR).

10035391Z6

Source: https://www.industrydocuments.ucsf.edu/docs/jyyb0000

Budget justification

The studies proposed in this application take advantage of several strains of rabbits and mutant stocks available at the Jackson Laboratory. These may prove of considerable value in studies of tobacco products.

Although a wide variety of spontaneous infectious and hereditary diseases have been found in the rabbit, tumors have been reported infrequently. However, only a few systematic studies have been conducted. Our studies reveal that lymphosarcomas and hemolytic anemia occur with high frequency in rabbits, but that the incidence, type, and development are greatly influenced by age, breed, and other constitutional factors. Clearly, studies with genetically controlled rabbits both supplement and complement studies with inbred mice. This unique resource of rabbits at the Jackson Laboratory must be maintained and made available to research workers elsewhere. The two strains of rabbits, WH and X, are extremely valuable for studies in oncogenesis but their exploitation has hardly begun. Strain III is an excellent strain because of its AHH-inducibility. Fortunately, we have the professional staff and talent essential to the studies that we propose. The financial support requested from the Council for Tobacco Research for maintenance and study of these rabbits is minimal, but is adequate when coupled with existing support. New stainless steel cages are required for replacement of worn-out galvanized cages.

There is no need, at present, to include in this budget salary provision for 100% effort contributed by the Project Co-Directors, because this proposal relates to work supported by NIH research contract PH43-67-744 from the National Cancer Institute and NIH resource grant RR 00251 from the Division of Research Resources of NIH.

1003539172

Source: https://www.industrydocuments.ucsf.edu/docs/...

TABLE 1: CHARACTERISTICS OF RABBIT STRAINS AT THE JACKSON LABORATORY AS OF 15 OCTOBER 1972.

STRAIN (Name & Symbol)	COLOR	PREDOMINANT GENOTYPE	ORIGIN	COEFFICIENT OF INBREEDING(1)	PREDOMINANT ALLOTYPE	MATURE WEIGHT (g)	OTHER GENES SEGREGATING
III (sublines)	Albino	cc AA E^dE^d	New Zealand White Castle '32	0.97	$a^1a^1b^4b^4c^7c^7$	3800	1, scoliosis(2)
(III mo)				(0.97)			
(III vo)		(rcrc)		(0.93)			
(III ep)		(ep/ep)		(0.96)			
(III ah)		(Ah/ah)		(0.93)			
(III wu)		(Wu/wu)		(0.90)			
(III Da)		(Da/da)		(0.90)			
(III Dw)		(Dw/dw)		(0.95)			
(III vp)		(Vp/vp)		(0.92)			
(III cd)		(Cd/cd)		(0.96)			
III_c	Albino	cc AA E^dE^d mpmp	Subline of III	0.90	$a^3a^3b^4b^4c^7c^7$	4000	
X	Sooty yellow	$C(c^{ch2})$ aa ee bb Hahа	Castle's small race	0.82	$a^3a^3b^4b^4c^7c^7$	2200	dk, ±21-, As
WH	Agouti	AA EE WHWH Lsls(5)	Rockefeller Inst. 1949. Crossed with strains X, III, etc.	0.72		2300	e, r^2
AC(3)	Black, recessive white marking	aa E^dE^d, du^du^d Acac(5), Sbsb(5)	Dutch	0.76	$a^3a^3b^5b^5c^7c^7$	2400	y, du^w, c^{7-}
ACEP(3)	Black, recessive white marking	aa E^dE^d VV du^w/du^w, epep	Dutch	0.92	$a^3a^3b^4b^4c^{7-}c^{7-}$	2400	hg, c, v
ACCR (B)(3)(6)	Albino	cc sasa	Dutch	Inbred	$a^3a^3b^5b^5c^7c^7$	2400	y_{7+}
(Y) (6)	Albino	cc sasa	Dutch	Inbred	$a^3a^3b^4b^4c^{7-}c^{7-}$	2400	c^{7+}
OS	Black, minimal recessive white marking	aa E^dE^d, du^du^d, Osos(5)	Dutch Rockefeller Institute 1948	0.84	$a^2a^2b^4b^4c^7c^7$	3200	hydrocephaly(2), du^w
AX	Chinchilla	$c^{ch3}c^{ch3}$ AA ww Axax(5)	Outcross of Chinchilla race V to strains III and X	0.68	$a^3a^3b^4b^4$	3500	du, bu, c^{ch2}, c; hypospadias
AX_{bubu}	Albino	cc bubu	AX strain	0.76	$b^4b^4c^7c^7$	3500	c^{ch3}, c^{ch2}, a^1a^3
A	Albino	cc	Miscellaneous	0.93	$a^3a^3b^4b^4$	2700	1
C	agouti or black	AA or aa	Miscellaneous	0.96		2800	b, c, du

1003539178

Source: https://www.industrydocuments.ucsf.edu/docs/jzyl0000

TABLE 2: GENES OF IMPORTANCE TO STUDIES OF CONSTITUTIONAL DISEASE BEING MAINTAINED IN THE RABBIT COLONY AT [illegible]

Disease and Gene Symbol	Disease and Gene Symbol	Disease and Gene Symbol
Achondroplasia (ac)	angora (l)	ataxia (ax)
dach (Da) (6)	furless (f)	buphthalmia (bu)
dwarf (Dw) (4)	rex^2 (r^2)	epilepsy (audiogenic seizures, ep)
renal cysts (rc)	satin (sa)	gamma globulin alleles, Aa^1, As^2, Aa^2
mandibular prognathism (mp)	adrenal hyperplasia (ah)	gamma globulin alleles, Ab^4, Ab^5
hypogonadia (hg)	chondrodystrophy (cd)	gamma globulin alleles, Ac^7, Ac^{7-}
lymphosarcoma (ls)		osteopetrosis (os)
hemolytic anemia (ha)		spina bifida (sb)

Lethal muscle contracture, hypognathia, splay leg, diminutive dwarf, and cleft palate occur sporadically in some strains and may be genetic.

For other gene symbols, see:

Sawin, P. B. 1955. Recent genetics of the domestic rabbit. Adv. Genet. 7:183-226.
Robinson, R. 1958. Genetic studies of the rabbit. Bibliog. Genet. 17:229-558.
Fox, R. R. 1972. Taxonomy and Genetics In Biology of The Laboratory Rabbit. S.W. Weisbroth, A.L. Kraus, R.E. Flatt (eds) Academic Press (In Press).

Footnotes:

(1) Maximum coefficient in the strain computed according to Wright's formula for coefficient of inbreeding (F). Inbreeding is by sib mating or as close to sib mating as possible consonant with maintenance of the specific lethal or semi-lethal genes (indicated by underlining) and an optimal reproductive capacity and viability.

(2) Level of penetrance is dependant upon environmental conditions.

(3) Sublines of the same Dutch stock obtained from Rockefeller Institute in 1948.

(4) Formerly symbolized dw, then recognized in the heterozygote (Sawin 1955 Adv. Genet. 7:183) and the symbol changed to Dw to reflect a semidominant.

(5) The ac, Da, os, sb, ha, ls, ah, cd, and ax genes are maintained in their respective strains by progeny testing of prospective parents. Homozygous transmitters are obtainable from the same test.

(6) The ACCR (B) and ACCR (Y) strains are also referred to simply as strain B or Y respectively.

(7) Formerly da, now recognized, by ear papilla, in the heterozygote.

1003539179

Source: https://www.industrydocuments.ucsf.edu/docs/jzyl0000